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EXPLORING A ROLE FOR THE SERINE PROTEASE PACE4 IN PAIN ASSOCIATED WITH EXPERIMENTAL OA

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Purpose: PACE4 belongs to the family of proprotein convertases that process precursor proteins into biologically active products through cleavage at paired basic amino acid processing sites, which is essential for proteolytic maturation of a wide variety of proteins, including growth factors, hormones, neuropeptides and zymogens. PACE4 is broadly expressed throughout the body, including high expression in the central nervous system. PACE4 reportedly removes the prodomain of pro-ADAMTS-4 and pro-ADAMTS-5 in osteoarthritic (OA) cartilage, thus activating these aggrecanases. Recently, a single nucleotide polymorphism in PCSK6, the gene that encodes PACE4, was reported to protect against pain in subjects with radiographic knee OA. In addition, PCSK6 null mice are resistant to acute pain in a battery of algometric assays, implying a functional role for PACE4 in pain generation. Therefore, we decided to evaluate the role of PACE4 in progression of joint pathology and associated pain-related behaviours in surgically induced murine osteoarthritis.

Methods: Destabilisation of the medial meniscus (DMM) surgery was performed in the right knees of 10-week old male wild type (WT) or PCSK6 null C57BL/6 mice. Mice were monitored bi-weekly for mechanical allodynia in the hind paw as a pain-related behavior, using von Frey fibers and the up-down staircase technique. Sixteen weeks after DMM, locomotive changes indicative of “movement-provoked pain”, including distance traveled and climbing, were measured by overnight monitoring on a LABORAS platform. At 16 weeks post DMM, mice were taken down and knees were collected for histopathology according to OARSI recommendations. In addition, 16 weeks after surgery, mice were perfused transcardially with paraformaldehyde; the spinal column was decalcified prior to spinal cord sectioning of the L3–L5 levels. Immunohistochemical staining with anti-Iba1 was used to examine microglia in the dorsal horn. Quantification of the number and morphology of Iba1-immunoreactive microglia was performed according to established methods in order to assess microglia activation, a process that is involved in maintenance of chronic pain.

Results: Joint histopathology at 16 weeks post DMM revealed a total joint score of 22 ± 1 in WT mice, and 23 ± 4 in PCSK6 null mice. Development and progression of mechanical allodynia after DMM surgery was very similar in WT and in PCSK6 null mice: both strains of mice developed allodynia in the ipsilateral hind paw by 4 weeks after DMM, and this was maintained for 16 weeks.

Sixteen weeks after DMM, WT mice display locomotive changes indicative of “movement-provoked pain”, including decreased climbing and decreased distance travelled overnight. In contrast, PCSK6 null mice did not show signs of decreased movement at 16 weeks post DMM. At that time-point, the dorsal horn of the spinal cord (L3–L5) in WT mice showed marked microglial activation, whereas this was not the case in PCSK6 null mice. Since microgliosis has been correlated to chronic pain in models of nerve injury, this may suggest that PCSK6 null mice do not develop chronic pain in this OA model.

Conclusions: In summary, by 16 weeks after DMM surgery, PCSK6 null mice developed OA pathology to the same extent as WT mice. In addition, knee joint pathology was associated with mechanical allodynia after DMM surgery to the same extent as in WT mice. In contrast, unlike WT mice, PCSK6 null mice did not develop locomotor changes that are indicative of movement-provoked pain. This was associated with the absence of microglia activation in the dorsal horn of the spinal cord. These results further support earlier findings that PACE4 may play a role in pain and justify further studies to analyze central mechanisms.

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PAIN PATHWAY ACTIVATION IN DORSAL ROOT GANGLIA AND DORSAL HORN IN A MURINE SURGICAL MODEL OF OSTEOARTHRITIS

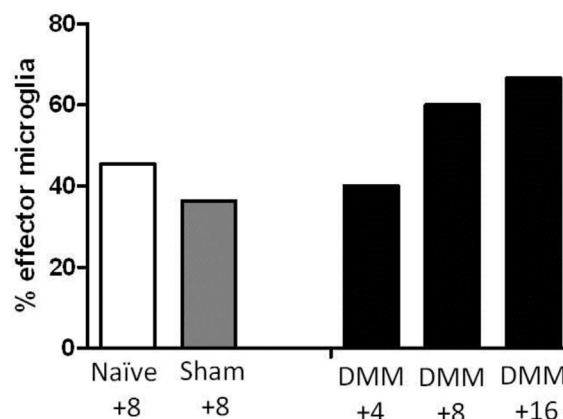
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Purpose: In nerve injury models, maintenance of chronic pain involves microglial activation in the dorsal horn (DH) of the spinal cord. This is a dynamic process, which depends on signaling molecules, such as the chemokine fractalkine (CX3CL1), which are produced in dorsal root ganglia (DRG) neurons and transported to the DH, resulting in activation of DH microglia. We monitor pain and associated pathways over a period of 16 weeks post destabilization of the medial meniscus (DMM) surgery in the mouse. This model of slowly progressive knee osteoarthritis is associated with changing pain-related behaviors and concurrent molecular changes in the innervating DRG over 16 weeks post surgery. Specifically, mice develop progressive mechanical allodynia over the first 4 weeks, while locomotive changes indicative of chronic pain first appear 8 weeks post DMM. The purpose of the current study was to investigate fractalkine expression in DRG neurons as well as microglial activation in the DH over 16 weeks following DMM surgery.

Methods: DMM or sham surgery was performed in the right knees of 10-week old male C57BL/6 mice. Four, 8, and 16 weeks post DMM and sham surgeries, L3–L5 DRG were harvested and cells were cultured for 4 days; supernatants were collected for fractalkine ELISA. For immunohistochemistry, mice were perfused transcardially with paraformaldehyde, and the spinal column was decalcified prior to cryosectioning. To assess microglial activation in the DH, spinal cord sections were immunostained with anti-Iba1 and quantification of the number and morphology of Iba1 immunoreactive microglia was performed according to established methods. Microglia in which process length was less than double soma diameter were classified as effector (i.e., activated) microglia while microglia in which process length was more than double the soma diameter were classified as surveyor microglia.

Results: At 4 weeks post DMM surgery, cultured DRG cells released similar amounts of fractalkine compared to age-matched naïve cells. At 8 weeks post surgery, DRG cells released elevated levels of fractalkine protein compared to sham and age-matched naïve DRG cells ($p < 0.01$), and by 16 weeks post surgery levels were still somewhat elevated in DMM cells ($p < 0.05$), but less so than at 8 weeks.

The dorsal horn showed increased Iba1 expression at 8 and 16 weeks but not at 4 weeks post DMM compared to sham and age-matched naïve mice.



At 8 and 16 weeks post DMM surgery, there was more effector microglia expression in the DH, particularly at the L4 level of the spinal cord (Fig 1).

Conclusions: The temporal correlation of fractalkine release with microglial activation suggests that fractalkine may contribute to microglial activation in the DH of the spinal cord. Microglial activation in the DH has been reported in murine mono-iodoacetate (MIA) arthritis as well as nerve injury models. Results from this study will help elucidate how pain signals propagate from the peripheral to the central nervous system in osteoarthritis.

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PRAGMATIC APPROACH TO QUANTITATIVE SENSORY TESTING IN KNEE OSTEOARTHRITIS: MEASURES OF MECHANICAL PAIN SENSITIVITY PREDICT CONCORDANT PAIN AND STRUCTURAL STATUS

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Purpose: While pain is the most important patient reported feature of OA, the pathophysiology of pain in OA is poorly understood with multiple mechanisms ranging from structural to neurogenic. Identifying pain mechanisms at a patient level will inform individualized treatment strategies and reducing the burden of OA.

Quantitative Sensory Testing (QST) is a psychophysical technique being increasingly used to assess patients with pain from osteoarthritis (OA). However, studies vary in terms of the number of repeats conducted for each individual test as well as the modalities used. To inform future research, this study aimed to:

1. Investigate the trends seen across subsequent repeats for each individual test, and
2. Examine which modalities are best able to distinguish between congruent and discordant pain and structural status.

Methods: 1003 women aged 43–65 years from a single general practice in Chingford, UK were recruited in 1988–9 for a longitudinal population-based cohort primarily for studying OA and osteoporosis. The current study includes data from the 20-year follow-up visit. QST modalities included warm detect threshold, heat pain threshold, mechanical pain threshold and mechanical pain sensitivity at the sternum (distant site) and knee (local site). All thresholds were repeated 5 times apart from mechanical pain sensitivity, which was repeated 3 times. Anterior-posterior, fully-extended, weight bearing, bilateral knee radiographs were taken and knee WOMAC scores were also recorded. The trends of individual repeats for each modality were explored graphically for each test site. Paired student *t*-test was used to identify any significant differences between adjacent repeats.

Subgroups were created using dichotomous splits of pain and radiographic osteoarthritis (ROA) status. Pain was defined as any pain captured using the WOMAC Pain subscore >0 and ROA was defined as Kellgren/Lawrence grade 2 or more creating 4 independent groups: ROA/pain, ROA/no pain, no ROA/no pain, no ROA/pain. Wilcoxon–Mann–Whitney and multivariate regression modeling, adjusting for clustering of sites within a person, were used to compare the association between QST measures and knee group. We adjusted for the following potential confounding factors HADS, age and BMI.

Results: In 462 women median age 71(67,76) years and mean BMI 27.7(5.0), 362 (39.6%) reported recent knee pain and ROA was present in 714/958 (74.5%) of knees assessed. Figure 1 demonstrates the trend for individual repeats seen at the sternum for each modality tested. The same trends were seen at the knee (data not shown). When comparing responses by group, mechanical pain sensitivity showed significantly increased experimental sensitivity at both local ($p = 0.0292$) and distant sites ($p = 0.0005$) in the ROA/Pain group compared to the ROA/No Pain group. A non-significant increased sensitivity was also seen in the No ROA/Pain group compared to the No ROA/No Pain group. These findings remained when adjusting for confounders using the multivariate regression model, Table 1.

Conclusions: These results suggest that three not five repeats are required for testing the QST modalities of heat pain threshold, mechanical pain threshold and mechanical pain sensitivity. For warm detect threshold the first measure should be omitted before calculating an average measure. Mechanical pain sensitivity was able to distinguish painful ROA positive knees from pain-free ROA positive knees. The fact that sensitivity at the sternum, as well as the knee, was able to predict concordant pain and structural status supports previous work showing that centrally mediated widespread pain sensitisation is present and highlights its potential use in future clinical research.

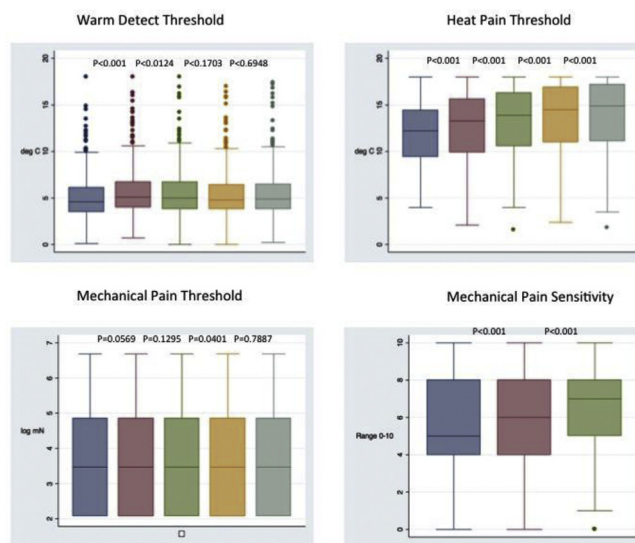


Figure 1. Trend for individual repeats of each QST measure at the Sternum

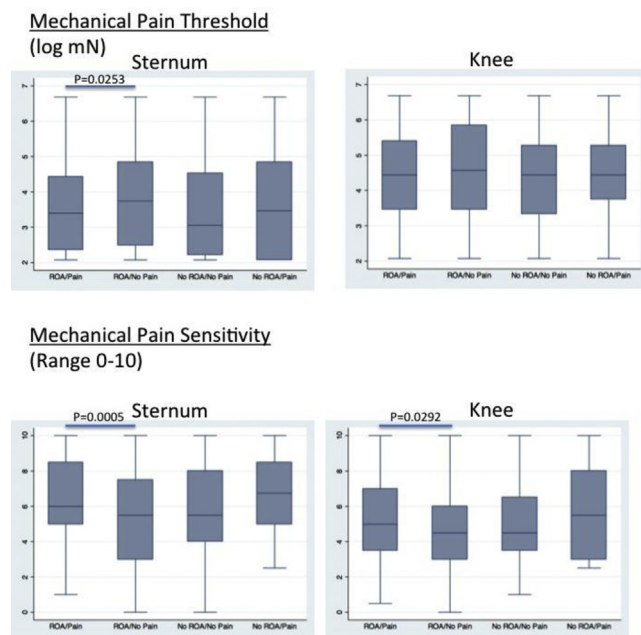


Figure 2. Group differences in Mechanical local and distant QST measures